MEMORANDUM

DEPARTMENT OF ENVIRONMENTAL QUALITY
WATER OPERATIONS

SUBJECT: Guidance Memo No. 98-2007
Evaluation of Calibration Curve Linearity

TO: Regional Directors

FROM: Larry Lawson, P.E.

DATE: September 16, 1998

COPIES: Regional Permit Managers, Regional Compliance and Enforcement Managers, Regional Technical Services Managers, Martin Ferguson, Richard Ayers, Bill Purcell, Betsy Ziomek

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SUMMARY: This document will replace the guidance of the same title dated September 18, 1996. Several changes have been made to the original evaluation guidance in order to more closely reflect current EPA guidelines and Standards for the National Environmental Laboratory Accreditation Conference (NELAC). The most significant change is the requirement for a calibration coefficient (r) of ≥ 0.995 regardless of the number of standards used to generate the curve. A lower r may be used only if specified supporting quality control samples have been analyzed.

The accuracy of analytical data is directly linked to the linearity of the calibration "curves" used to evaluate it. Calibration curves are derived by plotting known standard concentrations against an instrument measured response. For colorimetric analyses the response is measured with either a spectrophotometer or colorimeter measuring the absorbance or transmittance of a specific wavelength of light. For electrometric analyses, the response is measured in mV, or in some cases as a direct readout of the concentration. Using a calibration curve, an unknown analyte concentration can be derived from a sample by measuring it's response and then finding the corresponding y-axis intercept. Accurate results can be obtained when the relationship between concentration and response is linear. As the data becomes less linear, accuracy suffers. A curve can be constructed either graphically on paper, or mathematically using a computer program or calculator. It is easy to see how "good" a curve is when the best fit line is drawn between data points. However, obtaining results graphically can be misleading if care is not taken when drawing a curve or if the data itself is not absolutely linear.

Mathematically a best fit line can be drawn using the linear regression or least square analysis. Linear regression draws a best fit line that can be represented as:

$y = mx + b$

Where: (from the spectrophotometric example)

x = concentration

y = absorbance (or transmittance)

m = the slope of the line

b = y intercept

Manually calculating a linear regression is cumbersome. Fortunately with scientific and statistical calculators it is a simple matter of inputting x and y values to calculate a line of best fit. Once the slope and y intercept are known, it is easy to calculate a sample's concentration using the instrument's response for that sample. Keep in mind that the blank is not used when determining a linear regression. The blank is used for mechanically zeroing the instrument and therefore is not an instrument response.

Performing a linear regression doesn't indicate how tight the data is around the line of best fit. To determine this, the correlation coefficient (*r*) must be calculated. *r* is a method of statistically measuring how closely a straight line represents the data points of a xy graph. As with the linear regression calculations, manually computing the *r* value is a time-consuming exercise. *r* is easily determined, however, using a scientific calculator.

An *r* value of ± 1 indicates a perfect linear relationship between the data points and the best fit line. Positive correlation coefficients indicate a positive slope and negative values indicate a negative slope. It is worth mentioning that it is not critical which axis the concentration and instrument response are assigned to. Switching axis will cause the *r* value to change 'polarity' (+ or -), but the absolute value will not change. *r* is to be used only with data that is linear or data that can be transformed to behave linearly; e.g., log, reciprocal, square root, cube root, or square transformations. The general rule of thumb for an acceptable *r* is ≥ 0.995. This is in keeping with EPA guidelines and Standards for National Environmental Laboratory Accreditation Conference (NELAC). A lower *r* is allowed only if the laboratory has demonstrated consistent accuracy through the use of supporting quality control data (QC). This should include QC such as spikes, duplicates, check samples, MDL's, and **must** include a quality control sample prepared from a source different from that of the standards with a true value approximating the concentration of the samples being reported.

If inspectors are not familiar with these computations, the following tables contain "real" data with which they may practice. The data in Table 1 is for spectrophotometric total phosphorus analysis.

TABLE 1

Total Phosphorus		
Sample ID	Absorbance	Concentration
Blank	0.000	0.000
Standard 1	0.007	0.01
Standard 2	0.083	0.10
Standard 3	0.213	0.25
Standard 4	0.424	0.50
Standard 5	0.916	1.25
Blank Spike 0.25	0.207	102% Recovery
Sample 1	0.308	.795
Sample 1 0.25 Spike	0.501	105% Recovery
Sample 2	0.044	0.08
Sample 3	0.166	0.41
Sample 3 Duplicate	0.162	0.40
Sample 4	0.011	<0.01

The ***r*** factor for this calibration curve is 0.9977. Before practicing on this data set, keep in mind that there is a 2X dilution factor for the samples because 50 mL sample volumes were diluted to 100 mL. Standards were digested but not diluted. When evaluating this data set, one can be confident of the reported values because of the excellent correlation factor and supporting QC. Spike recovery indicates good analytical technique and the lack of matrix interference. Given this type of supporting information, an inspector would have latitude to overlook minor procedural discrepancies observed during an inspection.

Table 2 contains data from ion specific electrode (ISE) ammonia measurements. It is first necessary to perform a logarithmic transformation on concentration values before calculating the linear regression.

TABLE 2

ISE Ammonia		
Sample ID	mV	Concentration
Blank	+169.2	
Standard 1	+135.8	0.1
Standard 2	+97.6	0.5
Standard 3	+82.6	1.0
Standard 4	+45.2	5.0
Standard 5	+28.4	10.0
Standard 6	-24.8	100.0
Standard 7	-81.7	1000.0
Sample 1	+17.6	15.6
Sample 2	+107.3	.34
Sample 3	+97.5	.52
Sample 4	+80.8	1.1
Sample 4 Spike 5.0	+39.1	6.25 104% Recovery
Sample 5	+62.8	2.3
Sample 6	+83.7	.94
Sample 6 Duplicate	+83.5	.95
Sample 7	-24.2	92.2
Sample 8	+28.8	9.7

An ***r*** value of .9999 indicates a good xy correlation. The spikes and duplicate analysis show good recovery and precision respectively.

To illustrate the need to calculate a correlation coefficient with graphically determined data the following two data sets are offered. Table 3 contains data from a contract laboratory performing electrometric ammonia analysis.

TABLE 3

ISE Ammonia			
Sample ID	mV (x)	Concentration mg/L (from graph)	Concentration (y) mg/L (calculated)
Blank	-108.7		
Standard 1	50.3	0.1	
Standard 2	90.0	0.5	
Standard 3	107.3	1.0	
Standard 4	164.2	10.0	
Standard 5	204.9	50.0	
Standard 6	222.4	100.0	
ERA QC Sample 5.0 mg/L	151.3	5.4	5.8
Standard 5.0 mg/L	146.9	4.7	4.9
Sample 1	169.3	12	11.9
Sample 2	131.5	2.5	2.6
Sample 3	169.3	11	12.0
Sample 4	170.1	12	12.4
Sample 4 dup.	169.6	12	12.2
Sample 4 Spike 5 mg/L	180.2	17	18.6
Standard 5.0 mg/L	146.9	4.7	4.9

The curve used to draw this data set can be seen in Figure 1. The correlation coefficient is 0.99998. Note the difference between obtaining the analyte concentration from the graph versus from a linear regression. Values are close but some error is introduced by extrapolating off the graph. It is therefore suggested for inspectors to recommend analysts use linear regression rather than an actual curve to produce sample results. Most larger laboratories are currently using linear regression to calculate results because it is quicker and easier. It is not necessary to recalculate the regression for each sample run because the calculations are stored in memory. Standards and quality control samples (ERA) from a secondary source were analyzed throughout the sample run. The sample spike indicates no

matrix interference.

The data in Table 4 was prepared by the same analyst as the last example. Looking at the data it appears that meter and analyst were producing good results; unfortunately this was not the case.

TABLE 4

ISE Ammonia			
Sample ID	mV (x)	Concentration mg/L (from graph)	Concentration (y) mg/L (calculated)
Blank	-101.3		
Standard 1	54.7	0.1	
Standard 2	94.4	0.5	
Standard 3	111.7	1.0	
Standard 4	169.7	10.0	
Standard 5	209.2	50.0	
Standard 6	227.3	100.0	
ERA QC Sample 5.0 mg/L	156.1	5.2	5.9
Standard 5.0 mg/L	154.6	4.8	5.5
Sample 1	158.7	5.8	6.5
Sample 1 Dup.	164.5	6.4	8.2
Sample 1 Spike 5.0 mg/L	174.5	11	12.2
Standard 5.0 mg/L	156.2	5.1	5.9

The correlation coefficient for this six standard curve is 0.9999. Based on this information the curve shown in Figure 2 should produce excellent results, but this is not born out when graphed results are compared to results that are calculated using linear regression. Greater than 20% error is introduced in several of the results due to errors in either drawing the curve or extrapolating a value. The data in Tables 3 and 4 were produced with a linear curve by the same analyst, yet one set of results is acceptable and the other is not. Based on the ease and

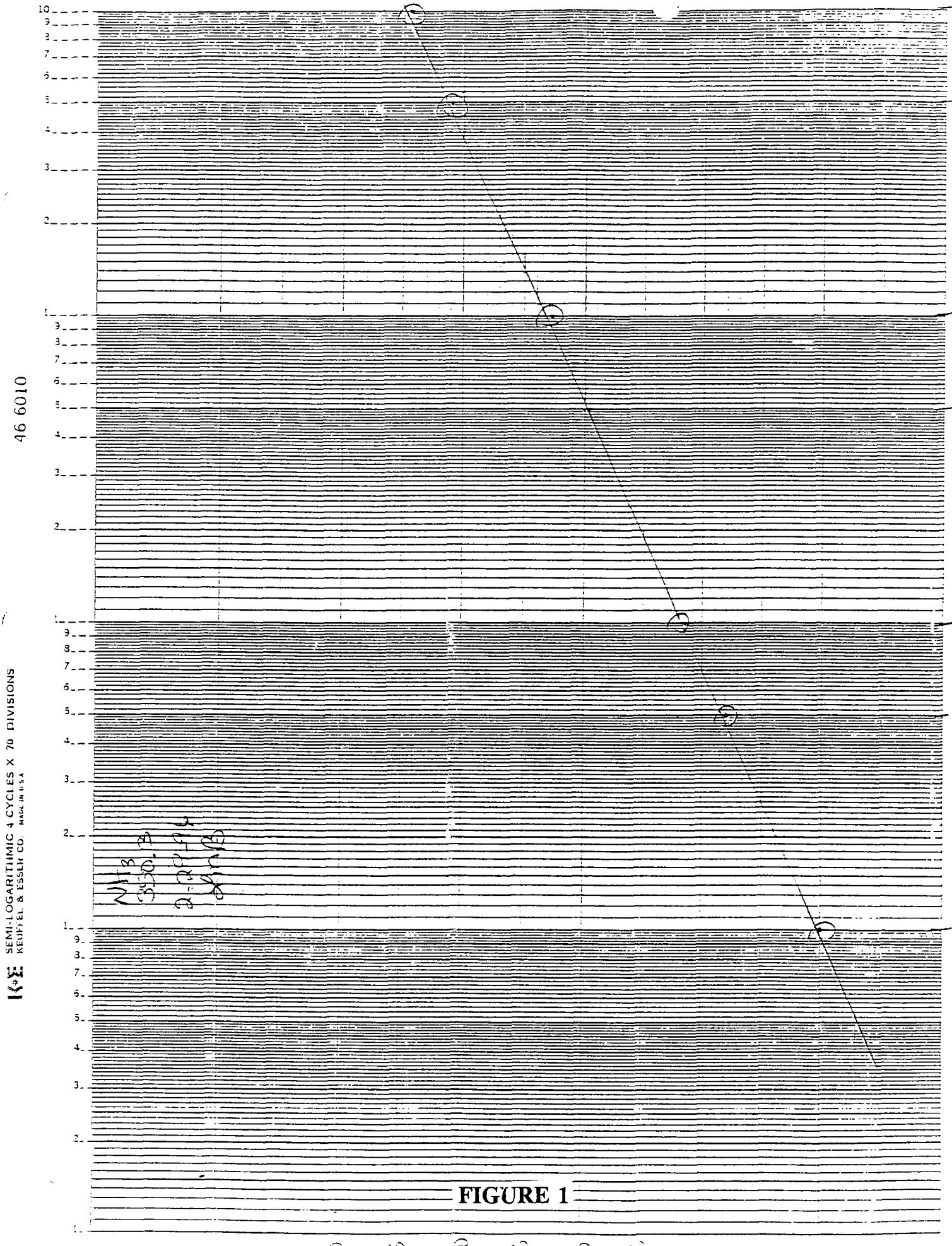
accuracy of producing results with the regression equation, inspectors should aggressively encourage analysts to use it.

Therefore, in the future, inspectors should review calibration curves to insure that the minimum correlation coefficient is met. This means ***r*** must be calculated for each new calibration curve created. Based on this guidance, if there is no supporting QC that validates the use of a < 0.995 ***r*** value, it will now be necessary for analysts to rerun standards until the minimum correlation coefficient is met. Analysts should be encouraged to either use direct concentration readout instrumentation or to calculate results using the regression equation. Inspectors should keep in mind that graphed results are still acceptable but can be expected to produce erratic results. Regardless of which method is used, the ***r*** value must be determined and noted on the bench sheet.

Questions or comments regarding this topic can be directed to Bill Purcell at (804) 698-4048 or Betsy Ziomek at (804) 698-4181.

DISCLAIMER

This document provides technical and procedural guidance to the inspection staff to evaluate laboratories producing data related to permit compliance. This document is guidance only. It does not establish a binding norm and is not finally determinative of the issues addressed. Agency decision in any particular case will be made by applying the State Water Control Law and the implementation regulations on the basis of site specific facts.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KEUFFEL & ESSER CO. MADE IN U.S.A.

FIGURE 1

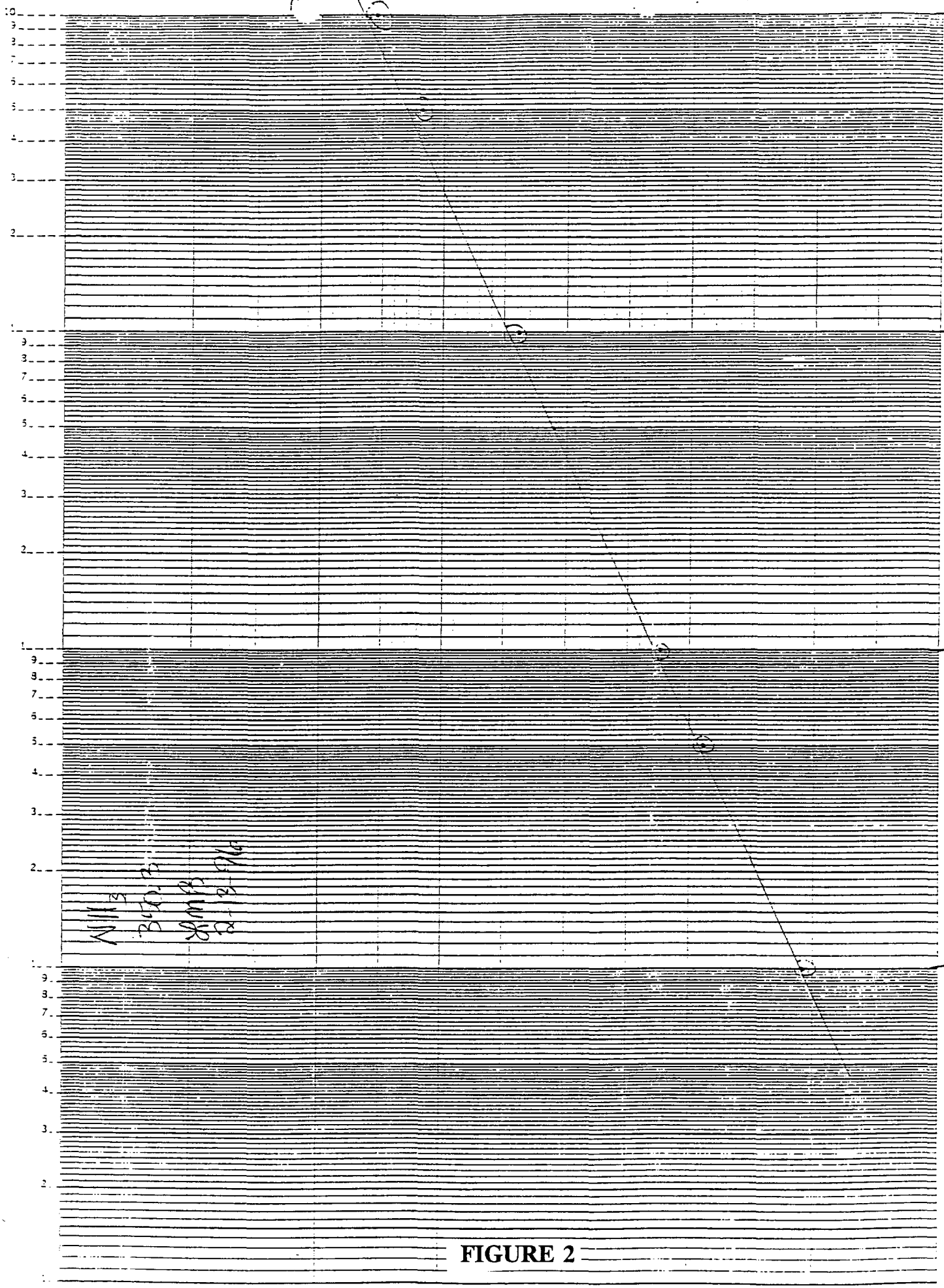

FIGURE 2